Physico-chemical and bacterial profiling of soils for describing a land-degradation gradient

Soil variables often show different response patterns to the same impact, making differences in soil quality a multidimensional property. Thus, analysing multivariate soil profiles often shows particular aspects of soil quality variation[1], and gives integrative soil quality measures such as a regression model[2]. Physico-chemical profiling of soils is relatively well established, while in recent years, biological profiling of soils has been popular[3]. The Biolog method[4] is used for biological profiling of soils, and provides community-level physiological profiles of soil bacterial communities. Profiling soils with the Biolog method may offer datasets different from those of the physico-chemical dataset[5]. We compared multivariate soil datasets provided by physico-chemical measurements and Biolog approaches[4,6] in describing a land-degradation gradient.

The Sakaerat Environmental Research Station, Thailand has dry evergreen forest (DEF, original vegetation), dry deciduous forest (DDF, moderately disturbed) and plantation plots as the major vegetative types. The vegetative types are distributed in a mosaic pattern in the northeastern part of the site. Bare ground (BG, most degraded), having no vegetation as a result of past human activities, is also scattered in the mosaic. DEF, DDF and BG soils were sampled on 4 November 2002, regarding vegetative mosaic as a completely randomized design[7]. At each sampling point, six soil cores were sampled to a depth of 5.1 cm, mixed in a plastic bag to make a composite, passed through a 2 mm sieve and then used for physico-chemical[7] and bacterial profiling. Values of a soil fertility index (SFI)[8] were calculated to quantify the intensity of land degradation.

The bacterial community was profiled with three Biolog EcoPlates. The above-mentioned composite sample, sieved and kept in a plastic bag at 5°C, was used 72 h after sampling. Five grams of the soil sample was suspended in 45 ml of sterilized water and reciprocally shaken at room temperature for 30 min at 120 rpm. The suspension was centrifuged at 1000 *g* for 5 min, decanted, and the pellet was re-suspended in 45 ml of sterilized water. Centrifugation and suspension were repeated twice. The soil suspension was left still for a minute, and 10 ml of the uppermost phase was diluted 40-fold with sterilized water. This suspension was used to inoculate the Biolog EcoPlates at a rate of 0.1 ml/well. The plates were incubated at 28°C in the dark and absorbance at 405 nm was read using an ELISA plate-reader at 5–16 h intervals for 8 days. During incubation, the plates were wrapped in a plastic film to avoid desiccation. Values for the above-mentioned three pseudo-replicates were averaged and used for the following statistical analyses.

All the statistical analyses were performed using SPSS 10.0.1 (SPSS Inc.). One-way analysis of variance was performed to test the effect of the land degradation on each soil physico-chemical characteristic. Dunnett T3 *t*-test was chosen as the *post-hoc* test. Kinetic parameters[6] were determined applying the criteria described by Mondini and Insam[9]. As a result, the number of variables was reduced from 31 to 22. The calculated values were ratio transformed; each value was divided by the sum of all the 22 values for the sample. The 22 variables were used for the calculation of average well colour development (AWCD)[4]. To compare the datasets in discriminating among the soils, the 22 ratio-transformed values were used for discriminant analysis of the community-level physiological profiles. Raw soil physico-chemical data were used for discriminant analysis of soil physico-chemical profiles. The putting independents-together method was chosen. Wilk's lambda statistic was calculated to quantify the difference among the soils[10]. Principal component analysis was performed to extract principal components from each dataset. Then, multiple regression analysis between the SFI values and the principal component scores was performed to obtain regression models for describing the land-degradation gradient. The stepwise method at the default criteria ($P = 0.05$ for inclusion and 0.10 for removal) was chosen. Simple linear regression analysis was performed to explore relationships between the scores on the significant principal component[11] (eigenvalue > 1) and the physico-chemical characteristics.

Most soil variables significantly reflected land degradation (Table 1). The DEF soil had the highest SFI value, followed by the DDF soil. The BG soil had the lowest SFI value, indicating that this soil was the most severely degraded. Land degradation represented by the vegetative types was a significant source of variation in SFI value.

AWCD values for the BG, DDF and DEF soils exceeded 1.2 after incubation periods of approximately 145, 126 and 122 h respectively, and then started to converge. The BG soil had the poorest total physiological activity[12]. Datasets for AWCD values of 0.6 and 1.2 were used as those at rapid and pre-convergence colour developmental time points.

Discriminant score plots are shown in Figure 1. The 1.2 AWCD and the physico-chemical datasets were the best for discriminating among the soils, regarding the low lambda values and the high significance. Differences among the soils were not clearly shown while analysing the datasets for parameters *K* and *S*. While analysing the datasets for parameter *K*, a DDF sample was misclassified as a BG sample, and for parameter *S*, a DEF sample was misclassified as a DDF sample. Analysing the other datasets resulted in no misclassification.

The first principal component derived from the physico-chemical data explained a large part of the variation, while that derived from the bacterial datasets explained smaller parts of the variations (Table 2). Significant principal components were: physico-chemical characteristics, 4; 0.6 AWCD, 5; 1.2 AWCD, 5; parameter *K*, 7; parameter *R*, 6, and parameter *S*, 5, suggesting more complex structures of the bacterial datasets.

Some minor principal components significantly correlated with the physico-chemical characteristics. For example, the third principal component derived from the 1.2 AWCD dataset significantly correlated with pH, exchangeable Al and H content and base saturation rate ($P \leq 0.01$). The first principal component derived from the bacterial datasets correlated with fewer physico-chemical characteristics than that derived from the physico-chemical dataset.

Scores on the significant principal components were used for multiple regression analysis. The following models were provided:

Table 1. Soil physico-chemical characteristics

Vegetative type	Moisture (%)	Bulk density (kg l^{-1})	Clay (%)	Silt (%)	Sand (%)	pH	Electrical conduti-vity (mS m^{-1})	Organic matter (g kg^{-1} dry soil)	Total nitrogen (g kg^{-1} dry soil)	Total carbon (g kg^{-1} dry soil)	Available phosphorus (Bray II) (mg kg^{-1})	Exchangeable cations K (c eq kg^{-1} dry soil)	Exchangeable cations Ca (c eq kg^{-1} dry soil)	Exchangeable cations Mg (c eq kg^{-1} dry soil)	Exchangeable cations Na (c eq kg^{-1} dry soil)	Cation exchange capacity (c eq kg^{-1} dry soil)	Exchangeable acidity Al (c eq kg^{-1} dry soil)	Exchangeable acidity H (c eq kg^{-1} dry soil)	Base saturation (%)	Soil fertility index
Dry evergreen forest	18.2[a‡]	0.97[a]	71.3[a]	15.9[a]	12.9[ab]	5.73[a]	11.3[a]	47.3[a]	1.83[a]	24.4[a]	8.28[a]	0.82[a]	2.83[a]	2.94[a]	0.14[a]	7.35[b]	0.23[b]	0.37[b]	91[a]	23.0[a]
Dry deciduous forest	16.8[a]	1.04[a]	75.3[a]	13.1[a]	11.6[b]	5.86[a]	7.3[b]	39.9[a]	1.22[a]	21.6[a]	4.10[b]	0.55[ab]	2.11[a]	1.84[ab]	0.13[a]	5.14[ab]	0.36[a]	0.57[a]	80[a]	14.8[b]
Bare ground	6.8[b]	1.40[b]	65.2[a]	13.8[a]	21.0[a]	5.00[b]	3.1[c]	19.2[b]	0.60[b]	8.2[b]	2.83[b]	0.34[b]	0.66[b]	1.05[b]	0.14[a]	4.18[a]	0.55[a]	1.44[a]	52[b]	13.5[c]
Analysis of variance[†]	0.000	0.000	0.108	0.402	0.024	0.000	0.000	0.000	0.000	0.002	0.000	0.001	0.000	0.004	0.421	0.004	0.001	0.000	0.000	0.000

[†]One-way analysis of variance was performed hypothesizing vegetative type to be the significant source of variation. *P* value for each soil characteristic is indicated.
[‡]Values in the column followed by the same letter do not differ significantly at $P = 0.05$ according to the Dunnett T3 *t*-test.

Physico-chemical,

$$\text{SFI} = 15.1 + 7.25 \times \text{PC1} + 1.42 \times \text{PC2} + 0.95 \times \text{PC3}, \; R = 0.979, \; P = 0.000, \quad (1)$$

0.6 AWCD,

$$\text{SFI} = 15.1 - 3.99 \times \text{PC4}, \; R = 0.524, \; P = 0.018 \quad (2)$$

1.2 AWCD,

$$\text{SFI} = 15.1 - 3.58 \times \text{PC3}, \; R = 0.621, \; P = 0.016, \quad (3)$$

Parameter R,

$$\text{SFI} = 15.1 - 3.62 \times \text{PC2}, \; R = 0.475, \; P = 0.034, \quad (4)$$

where PCn indicates a score on the principal component. No significant models were provided by analysing the datasets for parameters K and S. The physico-chemical dataset was the best for describing land-degradation gradient. Models for bacterial datasets did not contain the first principal component scores.

We attribute the suitability of the physico-chemical dataset for describing land-degradation gradient to the simple data structure (Tables 1 and 2). The simplicity was contributed by most of the physico-chemical characteristics having linear correlations with the first principal component (Table 2). Structures of the bacterial datasets were more complex than those of the physico-chemical data. The first principal component derived from the bacterial datasets was less significant than that from the physico-chemical dataset. Soil biological variables often have nonlinear response patterns to an abiotic environmental change[13]. Furthermore, multiple abiotic environmental changes affect a single soil biological variable, increasing its nonlinearity[5]. The nonlinear relationships were thought to contribute to the complex structures of the bacterial datasets.

Often, minor parts of a variation of multivariate soil profile explain a gradient of interest[14,15]. This was especially announced by the third principal component for the 1.2 AWCD dataset (Table 2). The third principal component explained the land-degradation gradient (eq. (3)), while the first and second principal components slightly correlated with the land-degradation gradient. Thus, compared with the physico-chemical dataset, fewer differences among the sample groups should be shown in the principal component score plot with the first and second principal components derived from the 1.2 AWCD dataset (Table 2). On the other hand, variation explained by the third principal component should have contributed to clear discrimination according to Wilk's lambda statistics, of which computation involves all parts of the variation (Figure 1). It is advantageous to find minor but meaningful parts of variation in data, as well as the most significant ones.

The altered community-level physiological profiles were the results of deforestation and the subsequent land degradation. The physico-chemical changes were also the results, while the relationships between the physico-chemical and the bacterial changes should be complicated. The hypothesis proposed by Oline and Grant[5] has its base on the assumption that abiotic environmental factors affect biological variables. The above results supported the hypothesis, and hence the cause and effect relationships seemed to be more influential than the reversed relationships. After the DEF was cleared and the soil was disturbed, the soil bacterial community was exposed to stress; high acidity[16], high temperature[17] because of the direct solar radiation and dryness[18] that can alter the original community-level physiological profile.

It is thought to be advantageous to apply the Biolog approaches together with the soil physico-chemical measurements. In an area, a unique integrative measure was provided surveying communities of plants that belong to a taxonomic group. The measure differed from those of other plant taxonomic groups in the same area[19]. Similar independence was reported among invertebrate taxonomic groups[20] and soil

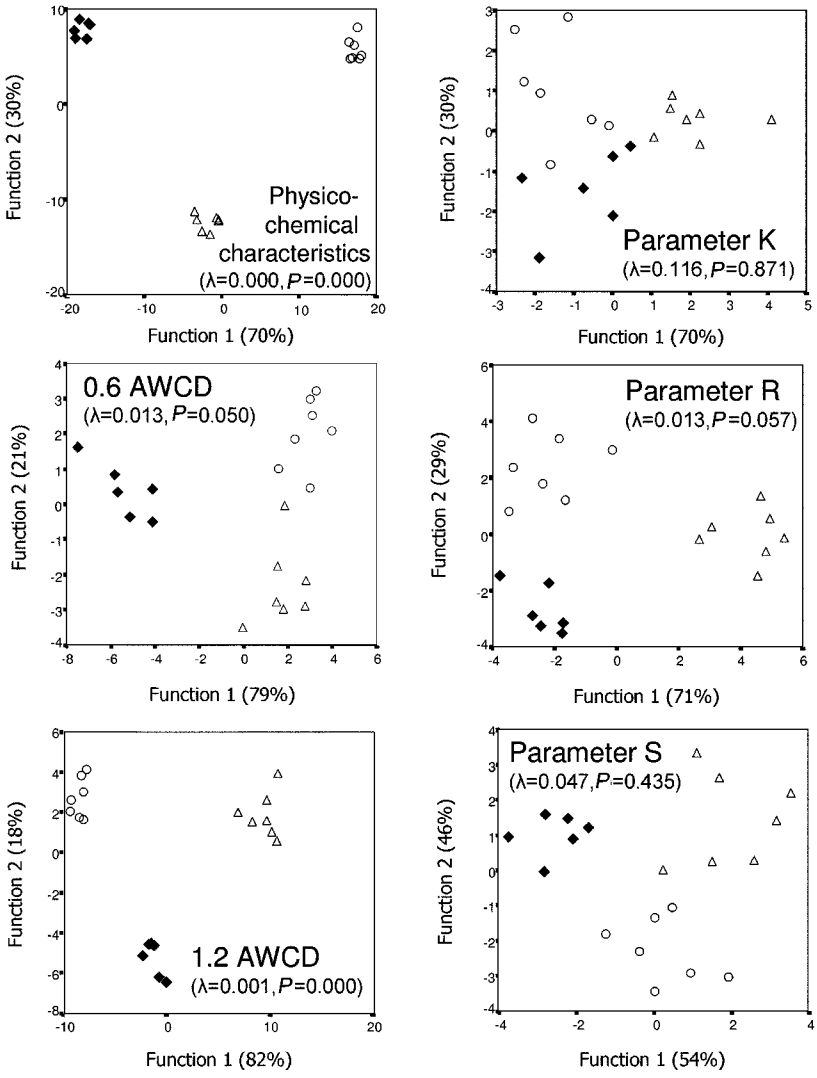

Figure 1. Discriminant score plots for datasets. Diamond, circle and the triangle indicate bare ground, dry deciduous forest and dry evergreen forest respectively. Percentage in parentheses indicates variability explained by the function.

Table 2. Soil physico-chemical characteristics that linearly correlated with significant principal components (eigenvalue > 1). Underlined physico-chemical characteristics had negative correlations with the principal component

Dataset	Principal components extracted from the dataset			Significant physico-chemical characteristics[‡]		
	Principal component[†]	Variation explained (%)	Eigenvalue	$P \leq 0.01$	$0.01 < P \leq 0.01$	$0.05 < P \leq 0.10$
Physicochemical	1	61.5	11.7	Moisture, BD, sand, pH, EC, OM, TN, TC, P, K, Ca, Mg, CEC, Al, H, BS	Clay	
	2	12.9	2.44	Clay, silt, sand	P	
	3	6.0	1.13	Na		
	4	5.6	1.07		Silt	H, BS
0.6 AWCD	1	42.9	6.86		Silt	Clay
	2	16.9	2.70		pH, EC, K, Ca, H, BS	Moisture, TC, Mg, CEC, Al
	3	11.5	1.84			Na
	4	7.7	1.23	K, Ca, CEC	OM, TN, TC, BS	Moisture, silt, P, Mg
1.2 AWCD	1	31.9	5.11	BD	Moisture	EC, OM
	2	16.7	2.67			Clay, silt
	3	14.9	2.38	pH, Al, H, BS	Sand, Mg	EC, Ca
	4	8.2	1.31		Moisture	
Parameter *K*	1	27.4	4.38			EC, Al
	2	13.9	2.23			
	3	12.2	1.96		P	
	4	10.8	1.73			K, Ca
	5	7.7	1.23		Sand, OM, Ca, CEC	BD, clay, EC, TN, TC, Mg
	6	7.3	1.17	Silt	Clay	Mg, CEC
Parameter *R*	1	21.1	3.38			Clay, silt
	2	14.1	2.25	EC	Moisture, BD, pH, TN, TC, Mg, Al, H, BS	OM, K, Na, CEC
	6	7.7	1.23			Clay
Parameter *S*	1	33.9	5.42			
	2	14.6	2.33	H	Al, clay	Moisture, sand
	4	9.3	1.48		EC	Moisture
	5	7.1	1.14		Clay, silt	

[†]Only the first and second principal components and those that had significant linear relationships with any of the physico-chemical characteristics were included.

[‡]BD, Bulk density; EC, Electrical conductivity; OM, Organic matter; TN, Total nitrogen; TC, Total carbon; P, Available phosphorus; CEC, Cation exchange capacity, and BS, Base saturation. K, Ca, Mg, Na indicate exchangeable bases, and H and Al exchangeable acidity.

microbial aspects[21]. It is difficult to predict which dataset would be the best for describing a gradient of interest. Therefore, we cannot but rely on an empirical approach to obtain such an integrative measure. The Biolog approaches and the physico-chemical measurements offer multiple datasets. Then, the current approaches may provide integrative measures for predicting a result, for which there are no experimental data. These empirical approaches for monitoring soil quality are expected to contribute to land conservation and management practices.

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Identification of molecular markers linked with differential flowering behaviour of mangoes in Andaman and Nicobar Islands

Genetic differences within and between geographic populations of an ecosystem are likely to be defined by the population-fluxing pattern as influenced by various ecological factors in the immediate past and historical pressure on the genome. The Andaman and Nicobar Islands in the Bay of Bengal (lat. 6°45′–13°41′N and long. 92°12′–93°51′E) comprise over 572 islands and rocks[1]. Due to the long history of cultivation in these islands, many cultivars of mango from both northern and southern India are known to exist. The varieties from northern India failed to flower in the absence of low temperature and low humidity. However, the varieties from southern India established due to their coincidence of the climatic requirement. Open pollination of these varieties resulted in many clones with differential flowering pattern due to the introgression of genes during hybridization. Most of these clones exhibited an erratic habit of bearing round the year. Flower bud differentiation takes place during May–June, August–September and November–December. Some clones exhibited multiple flowering while others though morphologically similar to the multiflowering clones, flowered only once[2].

In the existing context, a systematic and concerted effort to find the cause of differential flowering was found to be important. About 30 clones comprising 20 single-flowering and 10 multiple-flowering types were selected. Among them, three multiple-flowering local types (GL 1, GL 2 and GL 3), four parental single-flowering lines consisting of Neelam, Malgoa, Bangalora and Banganapalli and three local single-flowering types (HL 4, HL 6 and HL 12) were selected for intensive screening.

In the present study we report that the morphological character does not exhibit any variation between parental and open-pollinated clones. Qualitative and enzyme activities of these clones showed significant variation between them. This confirmed the influence of environment on flowering behaviour. However, the role of genic action on flowering cannot be ruled out. Thus to identify the influence of genes in flowering and molecular markers linked with the gene, variation at DNA level was analysed. Availability of reliable polymorphic markers often limits accurate estimation of genetic variation among individual populations[3]. Usual DNA-based techniques such as RFLP through Southern hybridization and use of micro-satellites are expensive; also use of latter required DNA sequence information[4]. PCR-based RAPD approach has been a handy and convenient alternative technique for investigation on genome mapping[5,6]. Genomic DNA was extracted from semi-mature leaves (1 g) by a modified CTAB method and purified by chloroform-isoamyl alcohol and RNAse treatment. In order to make a better representation of each clone, equal amount of DNA of 20 samples of each clone was pooled and the resulting bulked DNA samples were used for PCR–RAPD analysis. Quantified DNA was diluted to 20–40 ng/μl used for PCR–RAPD analysis. A set of 50 random decamer primers were selected from OPB, OPC, OPE, OPF, OPQ and OPX obtained from Bangalore Genei Pvt Ltd, Bangalore. Amplification was performed in 25 μl reaction mixture consisting of 40 ng genomic DNA, 10× reaction buffer with $MgCl_2$ 15 mM, 10 mM each of DATP, DCTP, DGTP and DTTP, 0.2 mM primer and 0.6U Taq polymerase (Bangalore Genei Pvt Ltd). PCR amplification was carried out on thermal cycler well blocks (MJ Research Inc., USA) in 0.2 ml micro-centrifuge tubes. The process was started with a 4 min initial denaturation at 94°C followed by 45 cycles of 1 min at 94°C for denaturation, 1 min at 37°C for annealing, 2 min at 72°C for extension and ended with a final 10 min extension at 72°C. Amplification products were maintained at 4°C until electrophoresis.

The reaction product was resolved by electrophoresis in a 1.5% agarose gel using 1× TBE buffer at 8 v/cm for 3 h. A 1 kb ladder (Bangalore Genei Pvt Ltd). All the 50 primers were tested at least twice for reproducibility of banding pattern and